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Article

Racemic serine is less soluble than pure enantiomers due to stronger intermolecular hydrogen bonds

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SUMMARY

The chemical and physical properties of amino acids determine the properties of the proteins they compose. Two of these properties include solubility and chirality. It is known that pure D- and L-serine are approximately eight times more soluble than DL-serine, their racemic mixture, but the reason is not clear. Our hypothesis is that the difference is related to the types of H-bonds that stabilize crystals of the pure serine enantiomers compared to their DL-racemic mixture. We tested the hypothesis by examining the structures of microscopic crystals and observed that the D- and L-serine formed platelets while the DL-serine formed prisms. We also discovered that when saturated solutions of D- and L-serine are mixed, DL-serine crystals form within seconds, accompanied by release of heat energy corresponding to -2.7 kcal/mol. This exothermic heat can be accounted for if a new type of hydrogen bond can form between the D- and L-serine enantiomers during crystallization that is absent in crystals of pure enantiomers. We confirmed this conclusion by examining crystal structures previously reported in X-ray diffraction studies of serine crystals. The significance of our observation is that release of heat energy during crystallization of a mixture of pure D and L enantiomers has not been previously reported and may be unique to the amino acid serine.

INTRODUCTION

Although hydrogen bonds are relatively weak compared to covalent bonds, they play essential roles in stabilizing biological molecules like proteins and nucleic acids. The type of hydrogen bond can markedly affect the properties of these polymeric molecules. Because the hydrogen bonds between guanine and cytosine in the DNA double helix are stronger than those between adenine and thymine, DNA with a greater content of guanine and cytosine also has a higher melting point at which the double helix comes apart to form single strands.

The chemical and physical properties of amino acids also strongly influence the properties of the proteins they compose. For instance, hydrogen bonds stabilize the alpha helix and beta sheet structures present in most protein molecules. Furthermore, highly soluble amino acids, like serine and proline, tend to increase solubility of a protein in water while less soluble amino acids, like phenylalanine and leucine, decrease the solubility of a protein in water because they are hydrophobic and reduce interactions with water.

Another important property of amino acids is chirality. A molecule is considered chiral if it cannot be superimposed with its mirror image. When a molecule is chiral, the molecule and its mirror image are called enantiomers of each other **(Figure 1)**. All amino acids, except glycine, are chiral. Because solutions of the two enantiomers of chiral amino acids rotate polarized light in opposite directions, they are called D and L from the Latin combining forms *dextro-* and *levo-*, which refer to the right- and left-handed direction of rotation of the amino acid respectively. With a few rare exceptions, all of the amino acids of living organisms are L-enantiomers (1).

Our study focused on an unusual property of serine, its solubility. L-serine and D-serine are equally soluble in water at 20°C (420 mg/mL, ref. 2); however, if the two enantiomers are mixed in a 1:1 ratio, the solubility of the DL-serine is reduced to 50 mg/mL (3). The aim of our study was to test the hypothesis that solubility is related to the crystal structure of DL-serine. To test this hypothesis, we crystallized L-serine, D-serine, and DL-serine and compared the crystals by microscopy. We also performed calorimetry, defined as the measurement of heat released by a reaction. We found that pure solutions of D- and L-serine release heat when they were mixed. The results confirmed that DL-serine does in fact have a different crystal structure from D- and L-serine, and that the reduced solubility was caused by the increased strength of a new hydrogen bond (H-bond) that formed between the D- and L-enantiomers when they are mixed.

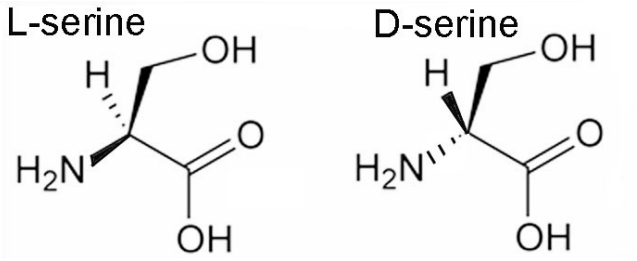

Figure 1: Molecular structures of enantiomers. The molecular structures of L- and D-serine are mirror images that cannot be superimposed and are, therefore, chiral. Note that the amine group in L-serine comes up out of the plane of the page while in D-serine it goes down into the plane.

RESULTS

Amino Acid Crystals

Our first goal was to test whether microscopy could be used to distinguish between different crystals of amino acids. Crystals of the L-enantiomers of asparagine, histidine, alanine, and glycine were grown on microscope slides as described in the Materials and Methods **(Figure 2)**. As expected, the microscopic structures were quite different because the shapes and chemical groups of the amino acid molecules varied and therefore the molecules assembled into different crystal structures. We concluded that our microscopic technique would be a useful way to examine crystals of serine both as pure enantiomers and as DL mixtures.

D- and L-Serine Crystals

The crystals of DL-serine were very different from the pure enantiomers **(Figure 3A)**. They were small, prismatic rods while the most obvious crystals of the D- and L-enantiomers were large rhomboidal plates that formed in the solution under the cover slips **(Figure 3B-E)**. The D- and L- enantiomer crystals were indistinguishable, as expected. The images in the figure were obtained both by phase microscopy shown in grayscale **(Figure 3B, D)**, and with polarization optics that added color for greater contrast **(Figure 3C, E)**.

Heat Released During Crystallization of DL-Serine

When saturated D- and L-serine were mixed, a mass of white crystals formed in a few seconds and the heat released increased the temperature of the mixture by 9°C. In order to calculate the heat of crystallization, we determined the mass of DL-serine crystals that formed. Before crystallization, the solution consisted of 4 mL of 1:1 D- and L-serine, 1.8 g of serine total. After crystallization, we expected that 0.2 g of DL-serine would remain in 4 mL of solution given that the known solubility of DL-serine is 50 mg/mL. The actual amount of DL-serine that precipitated as crystals was 1.6 g, which is similar to what we expected. The molecular weight of serine is 105 g/mol, so the amount that precipitated and gave off heat was 0.0152 moles. The crystallization heated the 4 mL of water by 9°C, equivalent to a total of approximately 36 calories. This amounts to ~2.7 kcal/mol of DL-serine crystals that precipitated. This result suggested that different H-bonds could form between the two serine enantiomers as they precipitated into crystals of DL-serine.

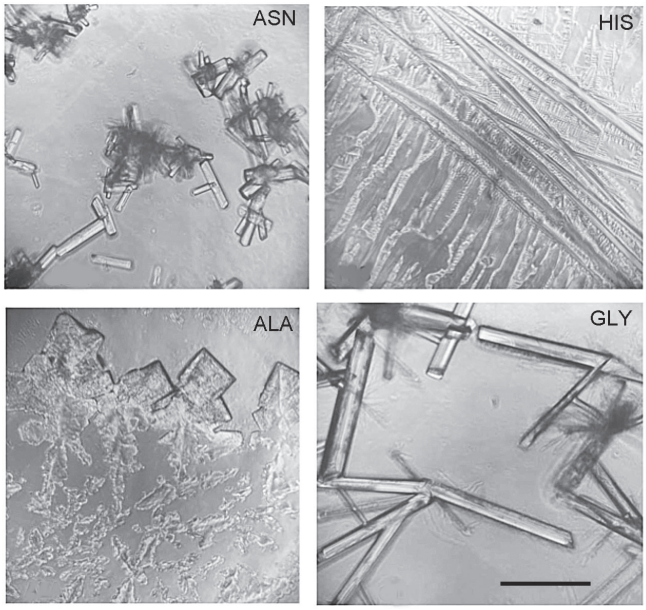

Figure 2: Crystals of four amino acids. The amino acid crystals shown are asparagine (ASN), histidine (HIS), alanine (ALA), and glycine (GLY). The crystals have different configurations because the amino acids have different molecular structures. 160X original magnification. Scale bar = 50 µm.

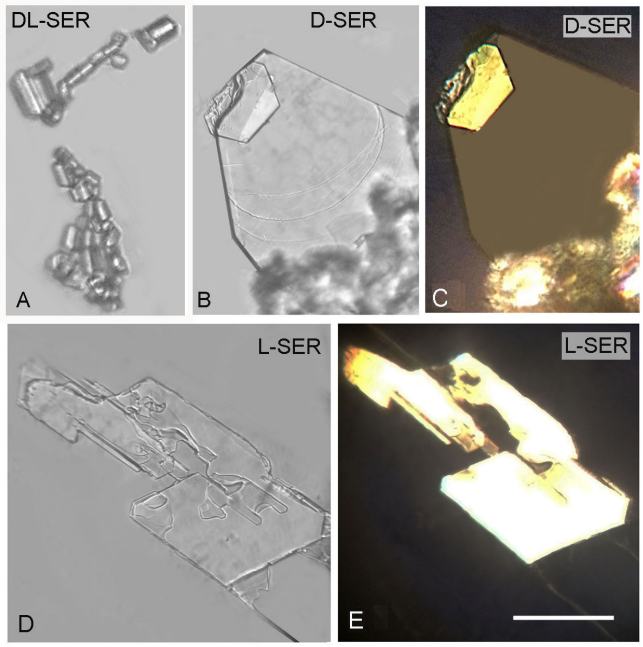

Figure 3: Crystals of DL-serine, D-serine and L-serine. Note that DL-serine formed small, prismatic structure (A) while D- and L-serine formed larger planar crystals (B-E). The color images (C, E) show the crystals of D- and L-serine using polarized illumination to increase visual contrast. Original magnification 160X. Scale bar = 50 µm.

DISCUSSION

Our results are consistent with the hypothesis that the difference in solubility of serine enantiomers compared to the solubility of the racemic DL mixture is due to differences in their crystal structure, perhaps related to different H-bonds that can form between the D- and L-enantiomers. The structural parameters of L-serine and DL-serine have been determined by X-ray diffraction and recorded in the Cambridge Crystallographic Data Center (CCDC) (4). L-serine is Space Group P 2_1 2_1 2_1 with cell dimensions of *a* 8.5213 Å *b* 9.172 Å *c* 5.5847 Å, α 90° β 90° γ 90° while DL-serine is Space Group P 2_1/a with cell dimensions *a* 10.739 Å *b* 9.149 Å *c* 4.830 Å, α 90° β 106.42(1)° γ 90°. D-serine was not listed in the CCDC

but is expected to be identical to L-serine.

It is clear that the structures are different, so we could now consider why mixing the enantiomers is exothermic. A clue to the answer was revealed by Jarmelo et al. who performed a theoretical calculation of the total H-bond energies in L-serine crystals (18.9 kcal/mol) and DL-serine crystals (24 kcal/mol) (5). This difference in total H-bond energies also accounts for the fact that the melting point of DL-serine is 240°C, while L-serine melts at 222°C.

Yu et al. performed an infrared spectral study of L-serine and DL-serine crystals (6). They found that in L-serine relatively weak H-bonds formed between the -OH groups on the molecules, while the molecular structures of D-serine and L-serine in DL-serine crystals allowed the -OH group of the L-enantiomer to form a stronger H-bond with the carboxylic group of the neighboring D-enantiomer as illustrated in **Figure 4**.

To summarize, when L-serine and D-serine are combined, they quickly form DL-serine crystals and release heat. The energy released from the temperature increase was equivalent to -2.7 kcal/mol. Jarmelo et al. reported that four H-bonds stabilize the crystal structure of both L-serine and DL-serine (5). In L-serine crystals one of the H-bonds is to the -OH group of its neighbor molecule, while in DL-serine the geometry of the molecules allows that -OH group to form an H-bond to the -COOH group on its neighbor, which is a stronger bond. The total calculated energy of the 4 H-bonds in the L-serine is 18.9 kcal/mol, and the total calculated energy of the 4 H-bonds in the DL-serine is 24 kcal/mol. The difference when they are mixed is 5.1 kcal/mol. However, our direct measurement is that heat released is equivalent to -2.7 kcal/mol when DL crystals form. It is uncertain why this value is less than the difference calculated from spectral measurements, but we note that Jarmelo et al. calculated the total energy for the formation of four H-bonds in the crystals (5). If we divide those values by 4, the energy difference per H-bond is 4.7 and 6.0 kcal/mol

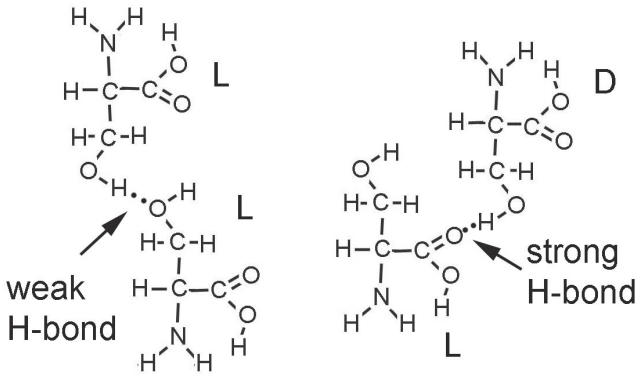

Figure 4: Hydrogen bonds in serine. The four -OH groups of L-serine in its crystals form relatively weak H-bonds with -OH groups of neighboring L-enantiomers. When a mixture of D- and L-serine crystallizes, one of the -OH groups can form a stronger H-bond with a -COOH group of a neighboring serine. This sketch is for purposes of illustration and does not represent an actual crystal structure.

for the pure enantiomer and DL-crystals respectively, while H-bond energies typically range from 2.4 to 4.8 kcal/mol. On the other hand, our measurement is an approximation which assumes that 1 degree of temperature change in 1 mL of water represents one calorie. It does not take into account that saturated solutions of serine in water might have a different heat capacity. Nonetheless, the increased bond strength of the H-bonds qualitatively explains why DL-serine is less soluble than D- or L-serine, and also accounts for the heat released when the two enantiomers are mixed.

MATERIALS AND METHODS

Microscopy

To test microscopic techniques, saturated aqueous solutions of several amino acids (Sigma-Aldrich) were crystallized on glass slides and examined with a Zeiss microscope using bright field and polarized light illumination. Crystallization was performed by adding 20 µL of saturated solutions to glass slides and covering the droplets with a cover slip. The solutions were made by adding amino acids to 2 mL of water until no more would dissolve. As the water evaporated over several hours, crystals formed around the edges and then penetrated into the solution under the cover slips where they were photographed several hours later at 160X magnification. D- and L-serine crystals were examined in the same way, and the crystals were compared to those of DL-serine.

Calorimetry

Two milliliters of saturated D-serine were mixed in a 5 mL polyethylene tube with 2 mL of saturated L-serine while the temperature of the mixture was monitored with an electronic digital thermometer. Polyethylene was used instead of glass because of its low heat capacity and the tube was insulated by a layer of glass wool. The thermistor probe of the thermometer was inserted into the mixture immediately before mixing. Crystallization began a few seconds after mixing and a white mass of crystals completely filled the test tube within 30 seconds, releasing heat during the process. The heat caused a temperature change that was monitored on the screen of the electronic thermometer. The experiment was repeated twice with similar results.

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